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A COMPARATIVE STUDY OF ASCOSPORE FORMATION BY 43 YEAST CULTURES[1]

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Abstract

In comparative tests for ascospore formation by 43 different yeast strains a solid medium containing acetate and dextrose was shown to be superior to two other sporulation media. Ascospores were formed more frequently and in greater numbers on the former medium. Eleven cultures of the thirty forming ascospores on acetate–dextrose agar yielded 50% or more asci, and seven cultures formed asci only on this agar medium.

Introduction

Recent advances in yeast genetics have stimulated interest in the phenomenon commonly referred to as "sporulation", but more precisely termed "ascospore formation", in yeast. A supply of ascospores, or of haploid cells resulting from the germination of single ascospores, is necessary in hybridization studies. Ascospores are also of prime importance in taxonomy. Over the past 70 to 80 years many methods and techniques for inducing ascospore formation have been described. Phaff and Mrak (6, 7) in a critical survey and review of the problem have discussed these methods. No single technique was found to give results consistently better than others with different yeast strains.

The selection of a particular method seems dependent upon the previous experiences of a worker with several methods which tend to establish a preference in favor of the least laborious procedure. The present work was undertaken to test with different yeasts the value of a simple method previously described by the writer (1). This involved the direct transfer of vegetative cells to an agar medium containing acetate and dextrose, and with bakers' yeast cultures it gave as high yields of ascospores as one of the more dependable methods—the Stantial–Elder liquid medium (3, 8). As a comparison, two other sporulation media were included in the tests, namely, water agar, a medium containing no nutrients, recommended by Beijerinck (2), and Gorodkowa's medium (4), which contains an ample supply of nutrients.

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Experimental

The yeast cultures selected included isolates of bakers' yeast, wine yeasts, yeasts isolated from plant tissues, aerial contaminants, and strains important for their yields of fat and protein. All strains were subcultured on "presporulation medium" twice before being transferred to sporulation media. Vegetative cells placed upon sporulation media were from actively growing 24 hr. subcultures. Two presporulation media for the multiplication of vegetative cells were employed, (1) Nutrient Agar (Difco) to which was added 5% dextrose and 0.5% tartaric acid, and (2) a medium composed of 2% agar and commercial grape juice, the latter containing approximately 5% grape sugar. Sporulation media employed were:

(1) Acetate–dextrose agar prepared by dissolving 0.04% dextrose and 0.14% anhydrous sodium acetate in distilled water and adding 2% agar. This medium was recommended in the author's preceding publication (1).

(2) Gorodkowa's medium (4), which contains 1% beef extract, 1% peptone, 1% agar, 0.5% sodium chloride, and 0.25% glucose in distilled water.

(3) Water agar prepared by the addition of 2% agar to distilled water.

For Experiment A, sporulation media were dispensed in test tubes as slants, and for Experiment B in Petri dishes.

The yield of asci was determined by preparing wet mounts from each tube culture, and in the case of the Petri dish cultures the yield was determined by direct observation of the cells upon the medium through a No. 1 glass cover slip. Thus each figure reported in Table I represents the average value derived from counts of at least 400 vegetative cells and asci taken from the center and outward to the edge of slant cultures or from different fields observed directly upon the surface of the media in Petri dish preparations. The sporulating cultures were examined at the end of 14 days' incubation at 20°–25° C.

Observations

As is shown in Table I, ascospore formation occurred most frequently and to a greater degree in cultures upon acetate–dextrose medium. Of the 30 cultures forming ascospores on this medium, 11 cultures yielded 50% or more asci, whereas no yield of more than 40% was observed from cultures on Gorodkowa's medium and seven of the same cultures failed to form ascospores on water agar. One culture (No. 10) formed asci on Gorodkowa's medium only while seven cultures formed asci only on the acetate–dextrose medium. The three bakers' yeast isolates readily formed four-celled asci, but only two of the other strains were able to do so. All cultures developed dense cell populations upon Gorodkowa's medium, whereas on acetate–dextrose agar the vegetative cells became more granular, losing the typical appearance of actively growing yeast cells as ascospore formation proceeded. Cells upon Gorodkowa's medium became less refractile and appeared paler than those on the presporulation media, while cells upon water agar more closely retained their original

TABLE I

ASCOSPORE PRODUCTION ON THREE SPORULATION MEDIA BY CULTURES MULTIPLIED UPON TWO PRESPORULATION MEDIA

Culture identification number**	Experiment A *		Experiment B *			No. of ascospores per ascus
	Percentage of asci after 14 days					
	Acetate-dextrose agar	Gorodkowa medium	Acetate-dextrose agar	Gorodkowa medium	Water agar	
1	90	20	90	20	5	1 - 4
2	90	2	90	10	5	1 - 4
3	90	20	90	20	5	1 - 4
4	5	Rare	5	Rare	0	1 - 2
5	7	Rare	5	Rare	0	1 - 2
6	21	Rare	10	Rare	0	1 - 2
7	65	10	70	5	40	1 - 2
8	15	2	20	2	1	1 - 2
9	5	Rare	15	Rare	0	1 - 2
10	0	0	0	Rare	0	1 - 2
11	20	2	25	1	1	1 - 2
12	50	4	40	2	5	1 - 2
13	40	12	35	8	5	1 - 2
14	60	14	40	5	10	1 - 2
15	45	20	50	10	10	1 - 4
16	25	0	20	0	0	1 - 2
17	Rare	Rare	Rare	Rare	0	1
18	70	40	65	35	65	1 - 3
19	1	1	1	1	0	1 - 2
20	45	25	50	25	35	1 - 3
21	1	0	1	0	0	1
22	0	0	0	0	0	
23	45	10	35	5	20	1 - 3
24	25	0	25	0	0	1 - 3
25	60	40	55	25	45	1 - 4
26	10	0	10	0	0	1 - 2
27	35	1	20	Rare	0	1 - 2
28	35	10	45	5	30	1 - 3
29	55	20	55	20	45	1 - 3
30	0	0	0	0	0	
31	25	0	25	0	0	1 - 3
32	70	0	65	0	0	1 - 2
33	60	0	75	0	0	1 - 2
34 - 43	0	0	0	0	0	

**The presporulation medium employed was grape juice agar in Experiment A, and glucose nutrient agar in Experiment B.*

***Cultures 1 - 3—bakers' yeast strains*
4 - 29—wine yeast strains
30 - 33—aerial contaminants
34 - 39—isolates from plant tissue
40 - 41—high protein producing strains
42 - 43—high fat producing yeast strains

appearance though exhibiting a tendency to swell, probably owing to the imbibition of water from the medium by the cells.

Discussion

Phaff and Mrak (7) found that the Stantial-Elder method gave a greater number of ascospores than did other methods or media in about 75% of the

Saccharomyces cultures studied, as well as giving good results with yeasts of other genera. However, they recommend fresh carrot wedges and, as an alternative, Lindegren's presporulation medium followed by transfer to gypsum blocks, while stating that "the Stantial method also gives excellent results but is tedious and, therefore, only recommended in cases of doubt and when other methods fail".

Gorodkowa (4), discussing the plaster block, stated "that this method requires a special carefulness and pedantry in work, and what is important, it is capricious and sometimes does not justify the trouble and the loss of time spent with it".* In fairness to the gypsum block method it should be understood that the effects of presporulation media upon ascospore formation had not at that time been fully recognized.

Lindegren and Lindegren (5) studied the effects of presporulation media composed of fruit and vegetable juices, singly and in mixtures, and have shown that the type of medium employed for the multiplication of vegetative cells may influence the number of asci formed by the cells when transferred to gypsum blocks.

The results tabulated here show that the acetate–dextrose agar medium is an effective substrate for inducing ascospore formation in a number of yeast strains. In view of the fact that this method is very convenient it may prove to be of considerable practical value.

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**Translation from Russian.*

USE OF MAGNESIUM ALLOY ANODES IN SHIP PROTECTION[1]

By K. N. Barnard[2]

Abstract

This paper describes the results of a 14 month test of magnesium alloy anodes in sea water. The current from the anodes was used to protect cathodically an inactive Algerine Class minesweeper. The results obtained show that it is practical to use magnesium alloy anodes in ship protection and that only a relatively small current is required to prevent the corrosion of underwater hulls of inactive ships. A convenient criterion to determine the amount of current required is given.

Introduction

The results of previous investigations (1) suggested that a more intensive use of cathodic protection should be the most effective means of preventing the underwater corrosion of steel ships in sea water. It was also shown that zinc, as normally applied, made an unsatisfactory sacrificial anode for ship protection. The zinc usually applied is Grade II and the presence of small amounts of impurities in this grade causes the anode corrosion products to form an adherent film. This film reduces the current output of the zinc anode to a small fraction of that value obtained when the surface is clean. The reduction in current output frequently takes place in less than six weeks. In addition, no special effort is made to ensure that the metallic contact between the zinc and hull is watertight, and the sea water seeping between the two surfaces soon destroys the electrical contact. The ineffective behavior of zinc anodes is illustrated in the photograph (Fig. 10) taken of a zinc anode fitted around an outlet in the ship's hull. It is to be noted that even after a year's service the edges of the anode are quite sharp, indicating only a slight consumption of zinc (i.e., small current output), and also that corrosion has occurred on the steel adjacent to the anode and on the retaining bolts.

In an effort to find a more effective sacrificial anode, the use of a magnesium alloy for the cathodic protection of naval ships has been under investigation for some time. The anodes were connected to an inactive Algerine Class minesweeper. Measurements were made of their current output and coulomb efficiency, and their general behavior was observed.

Concurrently the changes in the condition of the ship were followed by means of potential surveys, by "hull potential" readings, and by an inspection in drydock. The potential surveys were made using the method described in the previous report (1), and plots made of the differences in potential between two silver – silver chloride electrodes, one held next to the hull and the other held at a distance from the hull. The "hull potential" of a ship is the reading

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obtained when a high resistance voltmeter is connected to the hull and to a silver – silver chloride reference electrode held at a distance. Experiments have shown that if a current is applied to a steel plate so that the potential difference between the steel plate and a silver – silver chloride electrode is held numerically greater than 740 mv. (negative terminal of the voltmeter to the steel plate), the plate will not rust. This criterion was extended to the present experiment by means of the "hull potential" measurements.

This report describes the results of tests covering the initial period of 14 months. It shows that only a very small current is required for complete cathodic protection of a ship in reserve, and that it is practical to use magnesium alloy anodes to provide this current.

Magnesium Anodes

Excellent work had already been done to determine the best type of magnesium alloy for use in sea water by the Dow Chemical Company of America, and the Department of Mines and Resources in conjunction with the Dominion Magnesium Company of Canada.

These two companies donated samples of their commercial anodes for this experiment. The original three were supplied by the Dow Chemical Company and were all of different dimensions. Six from the Dominion Magnesium Company were all the same size, but the seventh was specially designed for use in ship protection. The dimensions of the anodes are given in Table I.

TABLE I

ORIGIN AND PHYSICAL PROPERTIES OF ANODES

Manufacturer	No. used	Type	Length, in.	Diam., in.	Cross section	Wt., lb.	% Composition by analysis		
							Al	Zn	Fe
Dow Chemical Company	1	1A	20	4	Circular	16	5.8	3.2	0.007
	1	4A	60		4 by 4 in. D Section	60	–	–	–
	1	5A	16	8	Circular	52	5.4	2.9	0.003
Dominion Magnesium Company	6		20	4	Circular	16	5.5	3.0	0.002
	1	Mk. I	48	6	Circular	93	5.4	3.4	0.001

The analyses of samples of the anodes were made by the Metallurgical Section of the Department of Mines and Resources.

H.M.C.S. *Wallaceburg*

H.M.C.S. *Wallaceburg*, a Reserve Fleet ship of the Algerine minesweeper class (length 225 ft., beam $35\frac{1}{2}$ ft.), was kept at a jetty berth throughout the experiments, except when the potential surveys were being conducted. The

ship had been drydocked from March 29 to April 3, 1947, during which time the hull was cleaned by hand and painted with two coats of iron oxide pigmented paint and one coat of antifouling paint. The water-line region (3 ft. wide) was chipped and scaled with pneumatic hammers, and prime coatings of zinc chromate and aluminum pigmented paint were applied. New zinc anodes were fitted in way of outlets and at the stern (Fig. 3).

For the first 254 days of the experiment, the ship was without heat; from then until the 322nd day it was continuously steam heated, either by an auxiliary vessel or by jetty steam, so that interior reconditioning and winterizing could be done more readily. A short length of rubber hose was used as an insulating link in the metallic steam line to prevent electrical contact between *Wallaceburg* and other ships, and the ship was secured by rope hawsers.

General Procedure

The magnesium anodes were suspended vertically from the port side of the ship by rope lines at an average depth of 16 ft. below the water line. A force fitting 1/2 in. bolt served both as a crossbar to which hooks for the suspension ropes were attached, and to provide a direct metallic contact between the commercial type of anodes and the heavy copper lead (size 19/052 in.). Rubber washers were used to retard water seepage between bolt and anode. In the Mark I design of anode, the steel crossbar and center rod were welded together, the magnesium cast on them, and the heavy copper lead was soldered directly to the center rod. The use of rubber washers was thus avoided. The lead from each anode or anode group was connected to the hull through a 0.02 ohm series resistance. Voltage readings were taken across this resistance to determine the current output of the anode, and a coulombmeter was used to obtain the efficiency of the Mark I type. The total metallic resistance between anode and hull was less than 0.05 ohm.

The anodes from the Dow Chemical Co. were suspended separately. During the experiment, one was consumed and another lost. After a time lapse they were replaced by Dominion Magnesium Company commercial anodes, connected in two groups of three. For the last 28 days the Mark I type also was used.

Frequent readings were taken of the current from each anode or anode group, and of the potential of the hull with respect to a silver – silver chloride reference electrode. Potential surveys, with and without anodes connected, were also made of the hull using the method outlined in a previous report (1). The anodes were disconnected by hauling them out of the water and laying them on the deck. This was also done whenever the ship was to be moved.

Results

Current Output

Current output curves for the various anodes are shown in Fig. 1. As was expected, these curves show the current output highest for the anode having

the largest area, and a general decrease in the current output of an anode over a long period of time. This decrease was not appreciable until a fairly large change in anode area had occurred. In fact, for anodes 1A, 4A, and 5A, the current increased with time over the period 80–119 days. This increase was most probably caused by an increase in water temperature (August–September).

The current output was greater when the ship was in Bedford Basin than when at its jetty berth. At the jetty berth the water was shallower, one side (starboard) was against the jetty wall and wave action was less. These factors would tend to give a lower current output. In addition, the anodes were disconnected for about two hours while the ship was being moved from its jetty to Bedford Basin–this would increase the current output. The minor irregularities in the curve were probably caused by changes in tide, wave action, salinity, and temperature. Of interest was the relatively large increase in the current output (approximately 15%) on the 120th day, after the anodes had been disconnected for three days, and the ship was in an unprotected state as indicated by its hull potential reading. This increase indicated that the current output of the anode was cathodically controlled to a fair degree. It was also found that the current output of two or more anodes was less when the anodes were held close together than when they were separated. None of the anodes were cleaned during this experiment, yet there is no evidence of a decrease in current output traceable to the formation of corrosion products on their surface.

Anode Appearance

The anodes were inspected periodically and were found to be of dull grey color (not metallic). Any corrosion film was very thin and of the order of 1/1000 in. The anodes were castings and apparently some segregation of the alloy metals had occurred at the original surface. This was indicated by the appearance of the original anode surface in relief after an immersion period of about a week. An analysis* of this part of the outer skin taken from the Dow Chemical anodes showed that it was rich in zinc and aluminum (Zn, 8.5%; Al, 8.4%; Mn, 0.1%). With time this skin (about 1/32 in. thick) was undermined and fell off, resulting in a slight increase in current output from the anodes. This was most noticeable for anode #5A.

The general appearance of this anode after 15 days and 162 days use is shown in Fig. 11A and 11B respectively, and it can be seen that, once the outer skin went, the anode was uniformly consumed over the entire surface except at a few spots. These spots were coated with a cathodic deposit, indicating that some local action had taken place on the anode surface with a resultant lowering of coulomb efficiency of the anode. The appearance and behavior of this anode was typical of all the anodes received from both companies. As a comparison the typical inert appearance of many zinc anodes after a year's service is shown in Fig. 10.

* *Made by the Department of Mines and Resources.*

Coulomb Efficiency of Anodes

The percentage coulomb efficiency was taken to be the per cent ratio of the observed coulomb output obtained from Fig. 1, to the theoretical coulomb output obtained from the observed weight loss, assuming the electrochemical equivalent for magnesium to hold for these anodes. Table II summarizes the weight loss, efficiency, position, and time of connection of the various anodes to the ship. The time of connection is given as the number of days after the beginning of the test on May 15, 1947.

TABLE II

ANODE EXPERIMENTAL DATA

Anode type	Position from bow, in ft.	Day connected	Day disconnected	Weight loss	% Coulomb efficiency	Remarks
Dow 1A	156	0	165	15 lb. 10 oz.	55	Steadily reduced in size until pencil-like stick remained
Dow 4A	160	0	261	?	?	Lost owing to action of ice and harbor craft.
Dow 5A	56	0	427	45 lb. 13 oz.	59	Still in use at end of experiment
Dominion Magnesium Co.	156 156	253 291	291 427	33 lb. 1 oz.	55	Two anodes were connected in parallel from 254th to 292nd day, when third anode was added in parallel
Dominion Magnesium Co.	56	268	427	31 lb. 9 oz.	56	Three anodes connected in parallel
Mark I	158	399	427	5 lb. 6 oz.	58	% Efficiency obtained from coulomb-meter readings

The values for the observed coulomb efficiency agree well with the manufacturer's claims. Actually the efficiency would be slightly higher, as the current between the magnesium and supporting steel hooks was not measured.

Hull Potential

In Fig. 2, the curve showing the "hull potential"—the potential difference between hull and silver - silver chloride reference electrode—indicated that the hull was cathodically protected for the first 165 days, except for a short period when the anodes were removed as part of a program to demonstrate their protective effect. For the period 220th to 270th day, the protection was inadequate. The protection improved on the latter day and, with the exception of one short period, the ship was adequately protected from the 300th to 362nd day. During this period, owing to the failure of the rubber insulating link, the hull was metallically connected to other berthed ships by the copper steam hose. This raised the current output of the magnesium anodes, but the increase

was not sufficient to polarize all the ships to the potential of *Wallaceburg*, hence the hull potential of *Wallaceburg* decreased. The hull potential again fell below the value required for protection before the 369th day; another anode was added on the 399th day, and the potential again increased to a protective value.

The criterion for the above statements on the condition of the cathodic protection of the ship is based on the results of experiments in seawater, showing that if the potential of a steel plate with respect to silver – silver chloride in seawater is greater numerically than 740 mv., the steel does not rust. In making the hull potential measurement, the anodes were frequently removed from the sea, and when this was done it was found, in general, that the hull potential reading decreased numerically by about 15–20 mv. from the value obtained with the anodes in the water. Taking this into account, a hull potential reading of 760 mv. may be considered the minimum value for complete cathodic protection with the anodes connected.

The influence of the magnesium on hull potential is well illustrated by the decrease in hull potential during the three day period following the removal of the anodes, 117th to 120th day. The hull potential dropped rapidly from 780 mv. to 645 mv., i.e., changed from a protecting to a rusting potential. After the anodes were reimmersed, approximately 30 days passed before the higher potential was regained; this indicated that the applied current was almost the minimum value necessary for full protection, and also that the polarization effects of such a current are slowly accumulative over a period of time. Several instances of this accumulative effect are noticeable on the potential curve, Fig. 2.

For the purposes of comparison, measurements were made of the hull potential of H.M.C.S. *New Liskeard*, an Algerine Class minesweeper in active service. The values obtained during the 4 to 10 months period after drydocking were all close to 600 mv., i.e., a rusting potential. Close-up surveys and a hull inspection (10 months period between drydockings) showed that the ship had been rusting, in fact much more rust was observed than for *Wallaceburg* after a 15 months period between drydockings.

Close-up Potential Surveys

An initial potential survey was made of the ship (May 15, 1947), prior to the connection of magnesium anodes. The results, given in Fig. 4, show that the hull was in a fair condition and that the zincs were still somewhat active at this date, six weeks after undocking. Those at the stern showed even as much as 60 mv. In general the negative potentials were small, but the anodic regions were confined to those near the zincs. Search electrodes held close to the propellers gave positive readings (30 mv.), and the potential gradient indicated that the current going to the propellers was relatively small. It was observed that the propellers were coated with a layer of weeds. The relative positions of underwater outlets and fittings around which zincs are fitted are shown in Fig. 3.

On completion of this initial survey, the magnesium anodes were connected and another survey carried out the following day. The results (Fig. 5) show much greater negative values, particularly along the water line, indicating that the ship was in a better state of cathodic protection. The strong positive regions on the port side were due to the positive field of the magnesium anodes, and the positive region at the stern was due to the effects of the zincs there. The current from the magnesium had appreciably reduced the field of the zincs. It was found in the next three surveys (May 27, July 11, and September 9) that these results were general; with time the anodic areas around the magnesium anodes increased in size and intensity as may be seen by comparing Fig. 6 (September 9) with Fig. 5.

On September 9 a second survey was made with the anodes disconnected. The results, given in Fig. 7, show increased anodic potentials, with the exception of the regions where the magnesium anodes were located. These regions, however, were still the most anodic portions of the hull, not because of more rapid rusting of the hull here, but owing to a polarization film formed by the action of the applied current. This is borne out by the survey made on September 12 (Fig. 8) which showed that the anodic action of the film was not present after the anodes had been disconnected for three days. The stern zincs were still somewhat active during this period but were quite inadequate to protect the hull. Another survey was made on the same day (Fig. 9) with the anodes reconnected, and the hull was again strongly cathodic, except for the regions at the magnesium anodes and at the stern near the zincs.

Surveys were made on the 125th, 216th, 253rd, 314th, and 369th day, all of which showed anodic regions near the magnesium and cathodic regions elsewhere. As to be expected, the intensity of the anodic and cathodic readings varied with the amount of magnesium used. On the 314th day, after disconnecting the anodes, a second survey was made. The results of this survey showed the same general characteristics as that of Fig. 7 made under similar conditions.

Drydocking Inspection

The ship was drydocked July 15, 1948, more than 15 months after the previous drydocking. Photographs were taken before and after cleaning the hull by hand using wire brushes; typical shots are shown in Figs. 12 to 15 inclusive.

Rusting of the ship was confined to five general regions, and covered a total area of less than 3 sq. ft. These regions were located as follows:

(1) On the damaged portion of the starboard bilge keel. The rust deposit was about 3/16 in. thick.

(2) Amidships, starboard side, about a foot below the water line. The several areas here were about six inches square surrounded by bare steel which had suffered no attack. The rust deposit was about 1/8 in. thick (Fig. 12).

(3) A single patch on the port side amidships, about a foot below the water line. Rust deposit less than 1/8 in. thick.

(4) Inside the asdic dome recess. The rust deposits here were scattered and nodular, about 1/4 in. diameter hemispheres. Exposed surface of the dome was free from rust.

(5) At the keel block positions unpainted at the previous drydocking. The rusting here had occurred only at a few isolated positions and it was merely surface rust, having no depth (Fig. 13).

With the exception of the rusting inside the asdic recess, all the rusting appeared old, and must have occurred during the time when insufficient magnesium was used (chiefly in the 165th to 270th day period), as indicated by the hull potential measurements.

No rusting had occurred elsewhere. All rivet points, both propeller shafts (Fig. 15) and "A" brackets, places very susceptible to corrosion, were free from rust. The paint on the propeller shafts had blistered and water had collected beneath the blisters, but the steel under the blisters was clean and shiny. Evidence that active corrosion had occurred prior to April 1947 could be seen in the pitted appearance of the rivet heads and shafts below the paint.

The zinc anodes were coated with a corrosion product. All the retaining nuts and bolts for the zincs were free from rust, in sharp contrast with their usual appearance after a period of a few months. It was estimated from the dimensions of the anodes that the original weight of zinc put on the ship in April 1947 was approximately 330 lb., of which 272 lb. remained at the July 1948 drydocking. The paint condition was better than is normal after 15 months' exposure for the type of paint used. There was some evidence of the descaling effect of the current along a narrow strip running from the bow to stern, about a foot below the water line on both sides, more noticeably towards the bow. Apparently a thick scale of old paint and rust had been present here. This had been loosened by the current and could be readily removed by chipping or brushing. Evidence of the loosening effect by the current was shown by the presence of a cathodic deposit below the scale. This deposit was readily removed by rotary wire brushing.

The fouling consisted of a general light growth of grass over the hull (Fig. 14), scattered clusters of mussels, and a growth of weeds near the water line. The paint was coated with a light layer of silt. The propellers, which had been observed to be coated with grass prior to connecting the magnesium anodes in May 1947, were badly fouled with mussels and starfish.

Competent naval authorities stated that the preservation of the underwater hull of this ship for the 15 month period was very much better than that of other ships under similar conditions of service. For instance, Fig. 16 illustrates the amount of corrosion that can occur on an inactive ship at Halifax, N.S., under normal shipyard treatment.

Discussion

All the above results indicate that the quantity of magnesium used, less than 180 lb., was almost sufficient to cathodically protect an inactive Algerine Class minesweeper for over a year. The effect of this protection was to decrease

markedly the amount of corrosion. The properties of the antifouling paint were not adversely affected, and the paint film was not stripped.

Owing to the effect of the sea path resistance at the very narrow entrance to the deep recess at the asdic dome, the current from the magnesium did not enter readily, and the current density at the inside steel surface (over 150 sq. ft.) was insufficient to protect the steel, even though zinc anodes had been fitted inside. For this particular recess it is recommended that the asdic dome be removed from reserve fleet ships to expose a relatively large opening, allowing free access for the protecting current.

The criterion used, i.e., a hull potential of 760 mv., to determine if the hull was rusting or not, was a simple one. The correlation between the graph of the hull potential versus time, the close-up potential surveys, and the drydocking inspection, show that it was effective except for the deep recess at the asdic dome. The reason for the failure to detect rusting here was the effect of the relatively high sea path resistance between the steel inside and infinity, compared to that between the hull and infinity.

Similarly the resistance of the sea path between the magnesium and infinity is high compared with that of the normally painted hull and infinity. In general the effect of instantaneously breaking the magnesium circuit was found to drop the hull potential only about 15 to 20 mv. If the hull was very well painted the effect of instantaneously breaking the magnesium circuit would be more marked, and it is for this reason that it is considered that the better criterion is the value of the hull potential obtained when the magnesium circuit is instantaneously broken.

On the basis of the results obtained with *Wallaceburg* it may prove possible for ships in reserve to safely extend the period between drydockings by using a sufficient quantity of magnesium, and still continue to follow the present painting procedure, instead of adopting the wet sandblasting technique and plastic paints. It is recognized that the better application of paints is desirable because it reduces the current requirements for cathodic protection, and extends the period between drydocking for the case of ships in active service, especially if supplemented by cathodic protection. But as the current required for *Wallaceburg* a year after drydocking was only the order of 15 amp., the saving in magnesium and the possible time extension may not warrant the more costly painting technique. This, however, must be decided by experience.

It must be kept in mind that these results were obtained from a ship in reserve, where it was practical to hang the anodes some distance from the hull—16 feet below the water line—for a continuous period. Further experiments are being carried out to determine the effect of securing magnesium and graphite anodes to the hulls of ships in active service, and the effect of the cathodic current on paint adhesion is being studied with those ships. In the meantime, however, it is strongly recommended that ships in active service carry sufficient magnesium anodes (type Mark I), to be slung over the side of the ship for protection when in harbor. It is estimated that for most naval ships this would afford complete protection for 50% of the time.

The drop in hull potential found to occur when the ship was connected to other ships or large metallic surfaces through a copper steam line demonstrates that, if cathodic protection is to be used, an insulating link should always be inserted in such connections. It also is suggested that an insulating link between ships and any common ground connection, such as steam lines, steel hawsers, etc., should be provided as a routine procedure, whether cathodic protection is applied or not.

A recommended design for magnesium anodes for use in reserve fleet ships is shown in Fig. 10c. This design provides a rod to which securing ropes can be readily attached and a convenient connection for the large copper lead going to the hull. The weight of magnesium per anode is slightly less than 100 lb.

Conclusions

The above results indicate that the outer underwater hull of a ship in reserve can be given adequate cathodic protection by the use of magnesium anodes located at two positions 16 ft. deep. The current required for protection in Canadian East Coast waters is quite small; an average value of about 1.5 ma. per sq. ft. would seem to be ample. This current requirement for an Algerine Class minesweeper could be provided by the consumption of about 220 lb. of magnesium per year.

The fact that the underwater hull was protected by the anodes at two positions is further evidence that anodes need not be placed at or near outlets, etc., as is the present practice, but may be positioned where it is easiest to secure them. For inactive ships this makes the installation of magnesium much more economical than the present methods of fitting zinc anodes directly to the hull at or near outlets. Moreover, material cost is also less and the adequate protection to be obtained should greatly decrease the cost of refits necessitated with the present shipyard techniques. The exception to this rule at present is the asdic dome recess. It may be necessary here to place an anode inside the recess, or to remove the dome, giving freer access to the interior, or to maintain the hull potential at a higher value in order to force more current inside the dome.

It should be practical to prevent the underwater corrosion of steel buoys, rafts, and active ships when in harbor by similar cathodic protection techniques, and, as the protecting current has no adverse effects on the antifouling properties of the paints normally applied, as shown with *Wallaceburg*, it also seems possible that active ships could be protected at all times by securing anodes to the hull without any increase in the rate of fouling.

The value of the hull potential is a good indication as to whether the underwater hull is rusting or not. A routine measurement of this potential can be made simply and a weekly check should be sufficient for a ship in reserve. For a ship in active service the measurements would have to be more frequent, since equilibrium conditions are not maintained over long periods, e.g., a ship is heavily loaded or in ballast, stopped, or steaming.

A ship when cathodically protected should not be electrically connected to other unprotected ships by metallic steam lines, wire ropes, or any other form of metallic contact.

Acknowledgments

This work was done at Naval Research Establishment, Halifax, N.S., as authorized by the Royal Canadian Navy and the Defence Research Board of Canada.

The author wishes to express his appreciation for the guidance of the late Dr. G. H. Henderson, O.B.E., F.R.S., formerly Chief Superintendent of the Naval Research Establishment, the co-operation of Captain A. F. Peers, O.B.E., R.C.N., Naval Liaison Officer, and the assistance of Dr. J. R. Dingle with the experimental program.

Reference

1. BARNARD, K. N. Can. J. Research, F, 26: 374-418. 1948.

[Figs. 1–16 follow.]

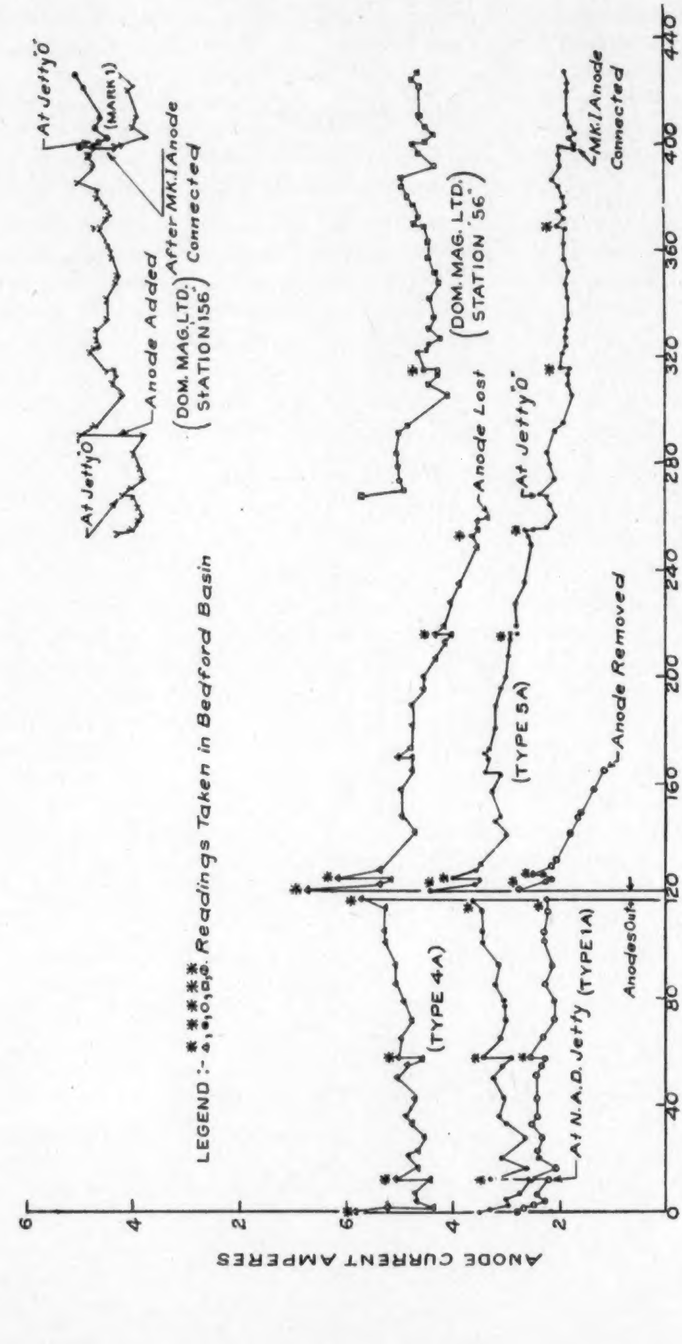


FIG. 1. *Graphs showing the variation of individual anode current with time.*

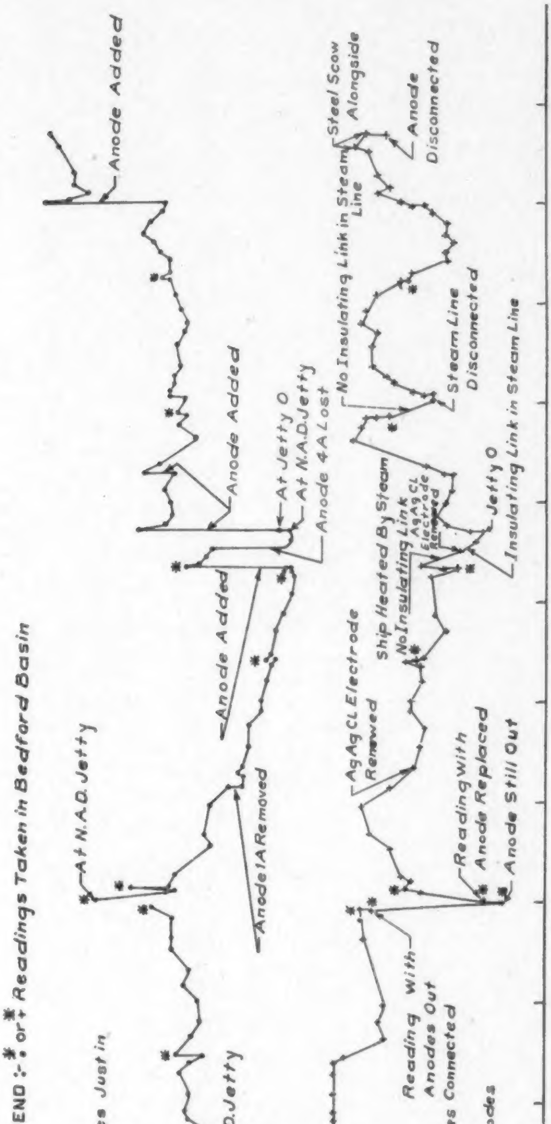


FIG. 2. Graphs showing the variation of total magnesium current and of the hull potential with time.

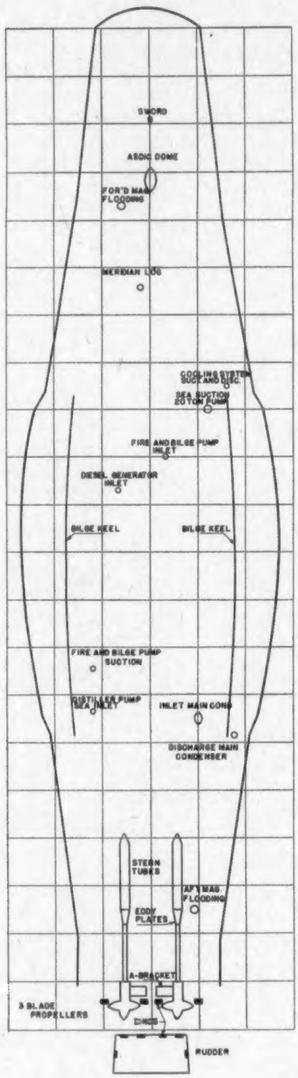
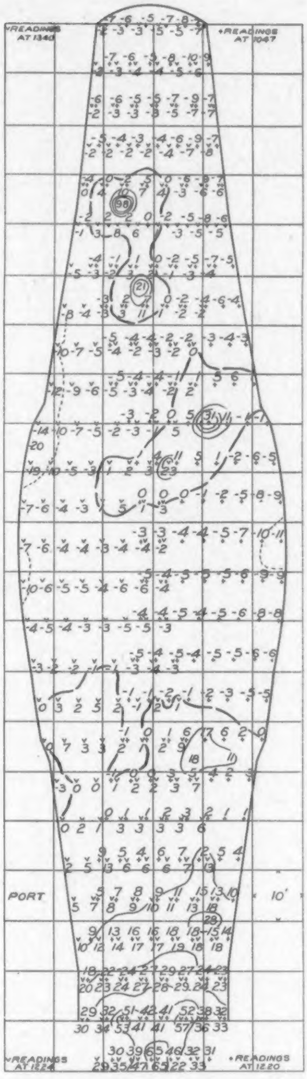


FIG. 3. *Outline of underwater hull showing position of underwater fittings.*



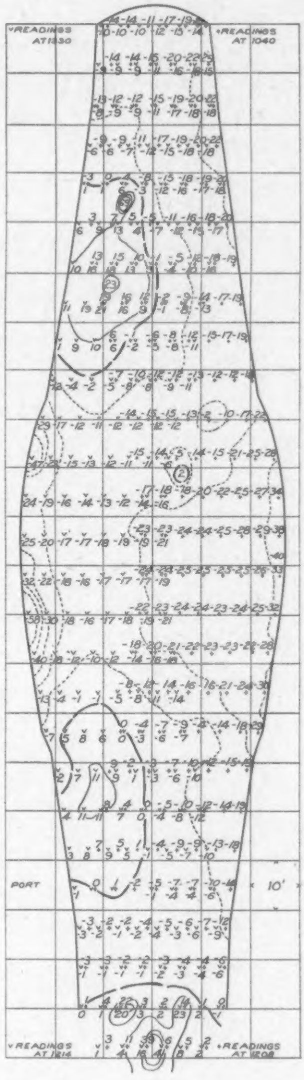
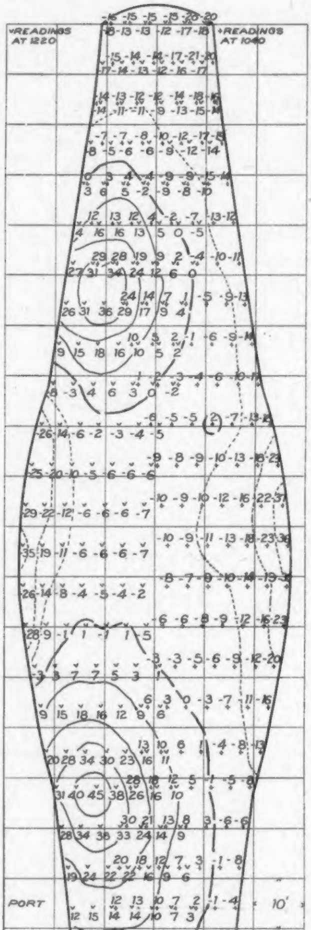


FIG. 4. *Results of survey on May 15, 1947.*
Weather — Clear, light wind.
Sea — Calm.
Remarks — Survey made just prior to addition of magnesium anodes.

FIG. 5. *Results of survey on May 16, 1947.*
Weather — Clear, light wind.
Sea — Calm.
Remarks — Magnesium anodes fitted May 15, 1947.



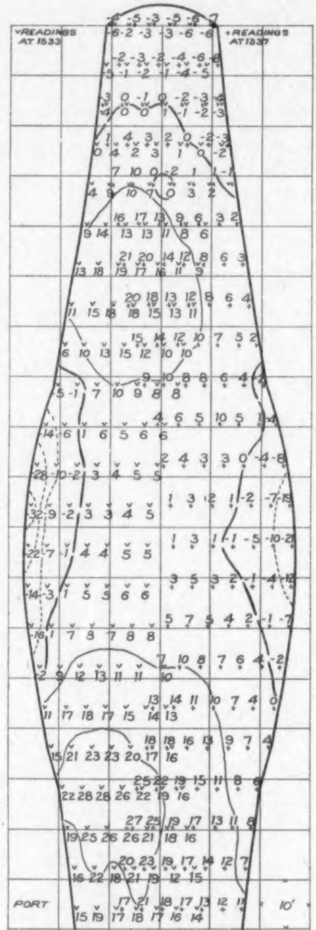
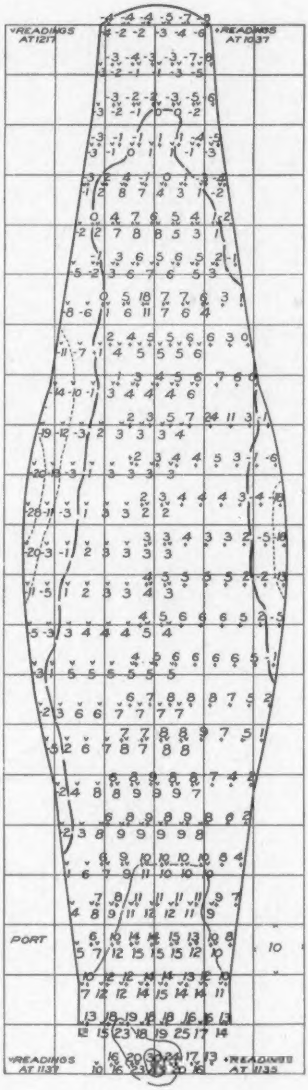


FIG. 6. *Results of 1st survey on Sept. 9, 1947.*
Weather — Clear, no wind.
Sea — Calm.

FIG. 7. *Results of 2nd survey on Sept. 9, 1947.*
Weather — Clear, no wind.
Sea — Calm.
Remarks — Magnesium anodes disconnected just prior to survey.



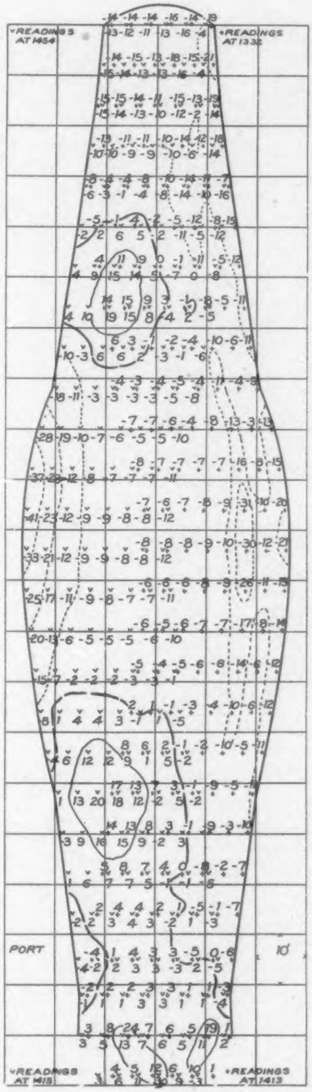
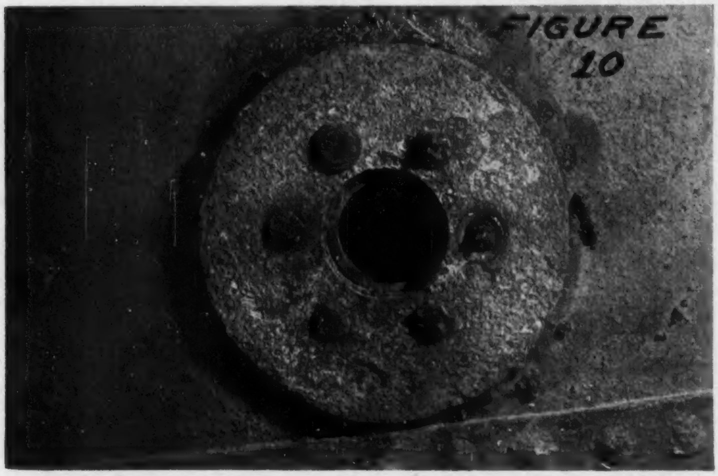


FIG. 8. *Results of 1st survey on Sept. 12, 1947.*
Weather — a.m. fog, p.m. clear, moderate wind.
Sea — 1 ft.–2 ft. waves.
Remarks — Magnesium anodes disconnected Sept. 9, 1947.

FIG. 9. *Results of 2nd survey on Sept. 12, 1947.*
Remarks — Magnesium anodes connected just prior to survey.

PLATE I



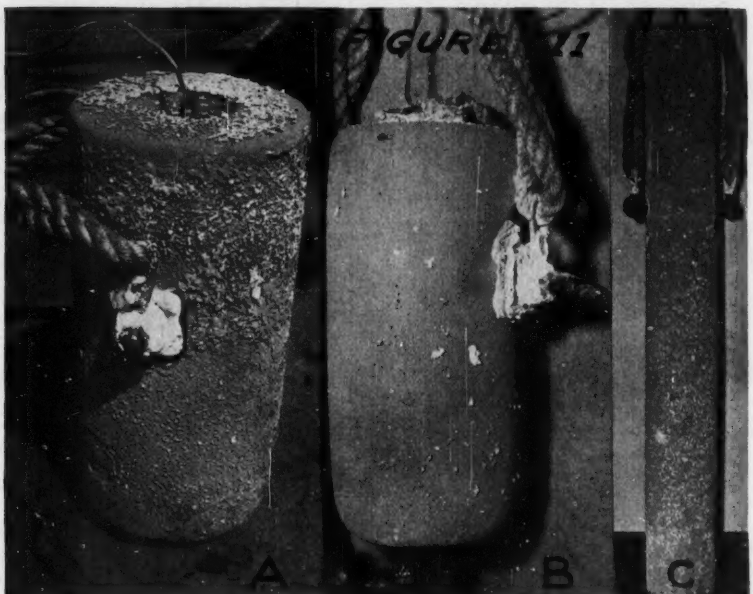


FIG. 10. *Showing the typical appearance of many zinc anodes after a year's service. Note the inert appearance of anode and the corrosion of securing bolts and the steel adjacent to anode.*

FIG. 11. *(A) The appearance of Anode 5A after 15 days' service. Note presence of anode skin.*
(B) Appearance of same anode after 162 days' service. Note absence of skin and the relatively uniform consumption of the anode.
(C) Appearance of Mark I anode after 28 days' service.

PLATE II

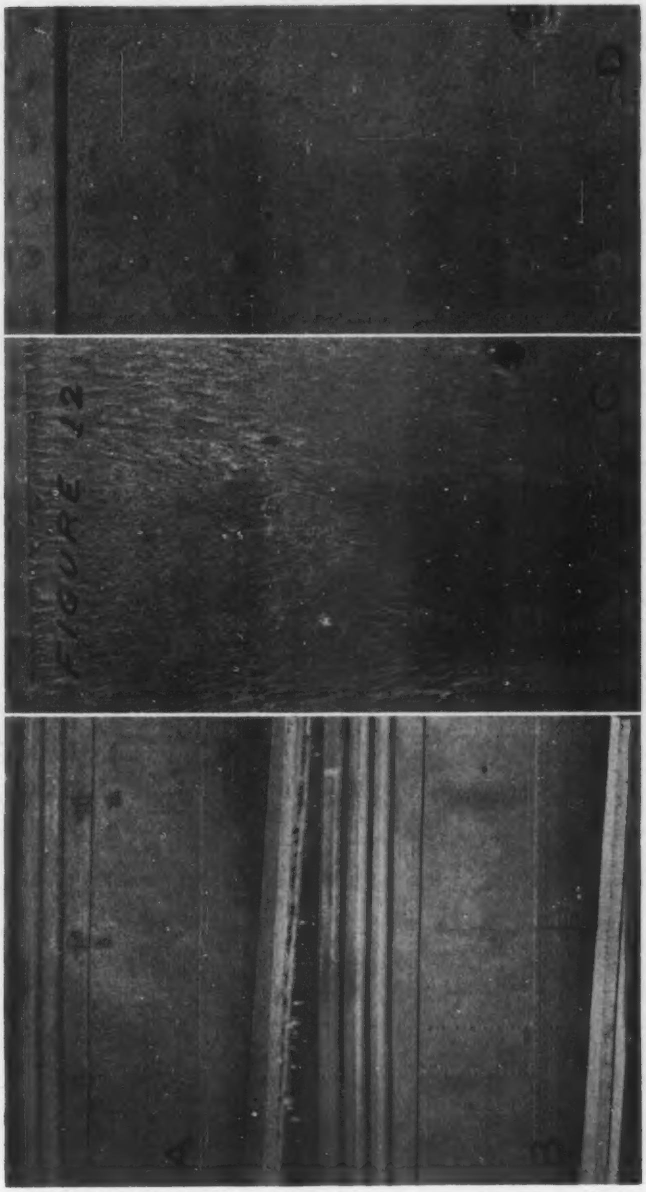
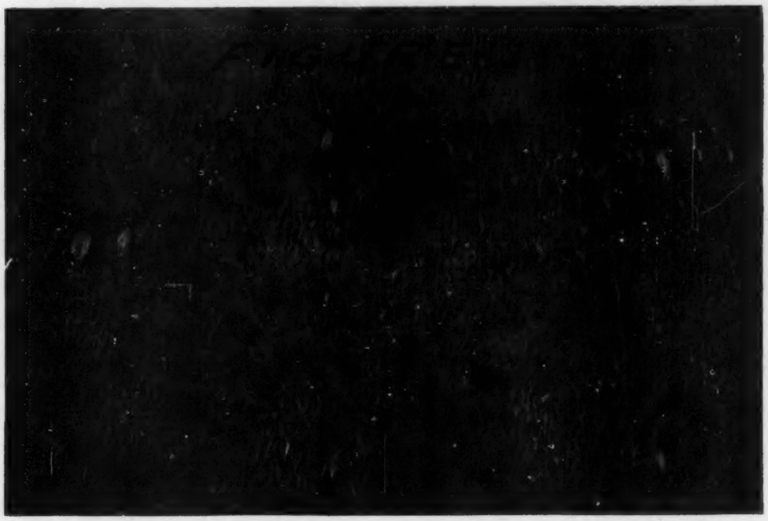


FIG. 12. *(A) Showing rust patches, starboard side amidships prior to brushing.*
(B) Same area after wire brushing by hand.
(C) Close-up of one of the rust patches prior to brushing.
(D) Close-up of same area after brushing.

PLATE III



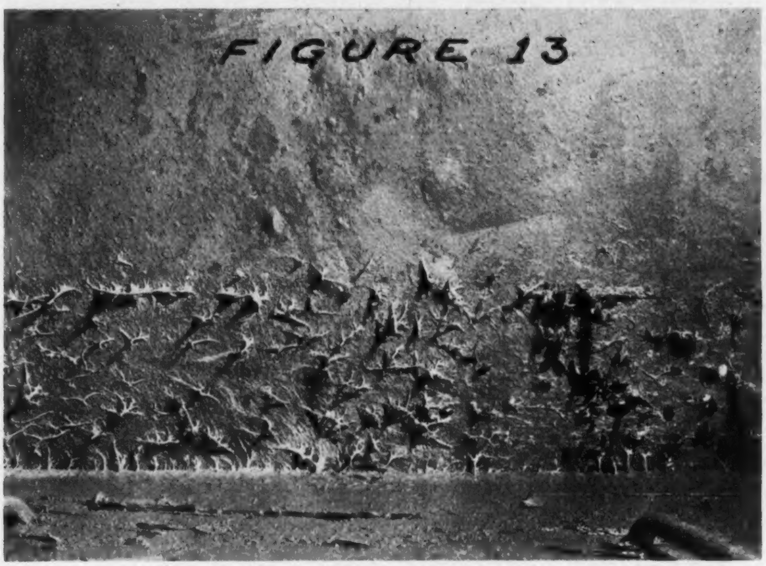
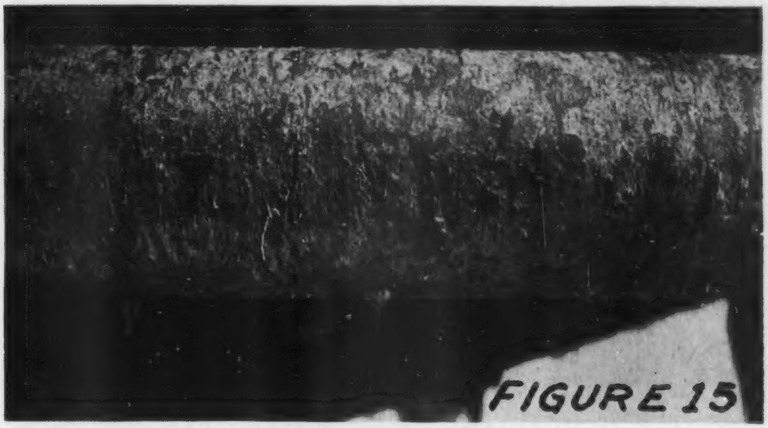


FIG. 13. *Typical condition of hull at previous keel block positions. Note absence of rust deposit.*
FIG. 14. *A close-up photograph of the general condition of the underwater hull, no rust and light fouling.*

PLATE IV



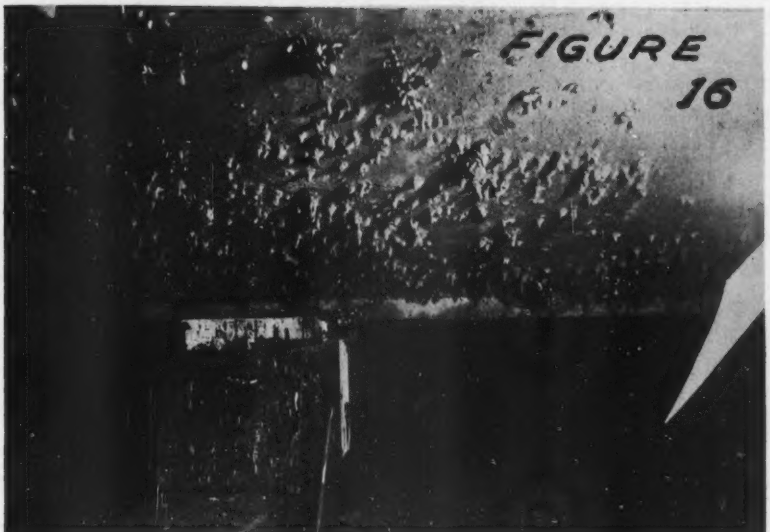


FIG. 15. *Close-up photograph of propeller shaft prior to brushing, no rust present.*
FIG. 16. *Fairly typical appearance of the underwater hull of a ship given normal shipyard treatment, after one year's inactive service similar to "Wallaceburg" 's. Note presence of numerous rust nodules approximately 1 in. at base and 1 in. long.*

EFFECT OF *ANTE-MORTEM* TREATMENT OF PIGS ON THE QUALITY OF WILTSHIRE BACON[1]

By N. E. Gibbons and Dyson Rose

Abstract

Meat from pigs slaughtered in a fatigued condition, as in present packing house procedures, had a lower glycogen reserve than meat from animals that had been fed and rested. Post-mortem breakdown of glycogen to lactic acid lowered the pH of meat from fed animals to approximately 5.3 while that from fatigued animals remained at about 6.0 or even as high as 6.6. Meat of low pH contained less sodium nitrite immediately after cure, and retained the desirable red color of cut lean surfaces much longer than did meat of high pH. Meat of low pH was also less susceptible to spoilage by bacteria. Color differences were less apparent after the meat had been smoked, and the preslaughter treatment of the pigs is therefore of less importance in the production of smoked products. The quality of unsmoked Wiltshire sides, on the other hand, would be greatly improved by proper preslaughter handling of the pigs.

Introduction

Experimental work during recent years has shown that the treatment an animal receives during the last 24 to 48 hr. before slaughter may markedly affect the quality of meat produced. Studies of the causes of "dark-cutting" beef have shown that the incidence of this complaint was reduced by proper preslaughter treatment (1). Callow and his fellow workers (7, 8, 13, 14, 17, 19) have studied several effects of preslaughter fatigue on cured pork products. Danish workers (21) have shown that feeding sugar a few hours before slaughter had a beneficial effect on bacon keeping quality and increased the liver weight.

Canadian packing plant methods differ in some respects from those used in Great Britain and Denmark, and the pigs themselves may differ considerably owing to different environment and breeding. Comparative studies on the effect of fatigue and feeding under the conditions normally found in Canadian plants were therefore undertaken, and the results obtained in three trials are presented in this paper.

Methods

Treatment of Pigs

Three trials were carried out using pigs received at the packing plant by rail or truck in the late afternoon. The pigs were assigned at random to treatment lots and held in pens with water until noon of the following day.

In Trial I, conducted in July, 1948, four lots of three pigs each were treated as follows: "Normal"—held overnight without food, then walked up a ramp 130 ft. long, and rising 26 ft., to the shackling pens; "Fatigued"—held over-

[1] *Manuscript received June 7, 1950.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as paper No. 252 of the Canadian Committee on Food Preservation and as N.R.C. No. 2235. Paper presented in part at the meetings of the Institute of Food Technologists, Chicago, May 22nd, 1950.

night without food and then driven to and fro along a passageway for 30 min. before being driven up the ramp to the shackling pen; "Rested"—penned at the top of the ramp overnight, without food; and "Fed"—given a total of 3 lb. sucrose mixed with 3 lb. barley chop per pig in two feedings 22 and 6 hr. before slaughter, but treated as "normal" pigs in other respects.

In Trial II (May, 1949) four lots of six pigs each were used. These lots were treated as in Trial I except that a total of 6 lb. sucrose and 6 lb. barley chop was fed. In Trial III (Sept., 1949) three lots of 20 pigs each were used and the "rested" treatment was omitted. The "fed" animals each received 1 lb. sucrose and 4 lb. barley chop.

The pigs were slaughtered and carcasses prepared in the normal manner except that, in Trials II and III, psoas muscles were removed before the carcass entered the cooler. In Trial I these muscles were removed from the carcasses after chilling overnight. The muscles from Trials II and III were brought to the laboratory and sampled for analysis as rapidly as possible, but at least two and up to four hours elapsed between the death of the animal and the preparation of the "initial" samples.

Analytical Methods

Muscle glycogen was determined by potassium hydroxide digestion, precipitation, hydrolysis (18), and reducing sugar estimation (24). Muscle lactic acid was determined in trichloroacetic acid extracts by the method of Barker and Summerson (3) modified to allow the use of the standard Evelyn colorimeter. In Trial II total extractable acids were determined by titration of a 24 hr. Soxhlet-ethanol extract.

The initial pH of the muscles was estimated by inserting glass and calomel electrodes into a slit cut longitudinally along the muscle fibers. The final pH was determined in 5 gm. of ground sample which had been mixed with 10 ml. of water plus a few drops of chloroform and allowed to stand 1½ to 2 hr. (14).

Chloride and nitrite were determined on hot water extracts of cured samples by the silver nitrate and sulphanilic acid-α-naphthylamine methods (26) respectively. The rate of penetration of salt from the curing pickle into the muscle was estimated by determining the chloride content of psoas muscles that had been immersed in brine for 24 hr. All surface fat and adhering tissue was removed from the muscles before curing, and an excess of pickle (meat to pickle ratio = 1:4) was used. After cure the muscles were ground and thoroughly mixed before the samples were removed for analysis.

Viable bacterial counts were made from dilutions in 3% salt solution plated on proteose peptone, tryptone agar containing 3% salt. Meat samples (5 gm.) were weighed aseptically and ground in a mortar with sand. Surface samples were taken by swabbing the surface and were diluted sufficiently in 3% salt solution to enable counting. These counts are proportional only. All plates were counted after seven days incubation at 70°F.

For morphological studies all of the colonies from a sector of the plate were transferred to slants, and Gram-stained preparations of 72-hr. cultures examined.

Results

Electrical Resistance of Muscle

The electrical resistance of pig muscle has been found (13, 17) to vary considerably with the *ante-mortem* treatment of the animal. This characteristic of the meat was determined in Trials I and II of the present work and average results in general agreement with those of Callow (13) were obtained. However, the variations between individual pigs were so large that the data were of little value and are not reported.

Liver Weight

An increase in glycogen content of the livers of fed animals should be accompanied by an increase in total liver weight. From a practical standpoint this would be of considerable importance as it would represent a direct return for the expenditure on feed. Livers from the animals used in Trials I and III

TABLE I

AVERAGE WEIGHT OF PIG LIVERS

Treatment	Trial I (3 pigs per treatment)		Trial III (20 pigs per treatment)
	Fresh wt., oz.	Fresh wt., % of dressed carcass	Fresh wt., oz.
Fatigued	45.0	1.87	51.5
Normal	43.3	1.88	61.1
Rested	49.7	2.13	—
Fed	58.6	2.59	66.5

were weighed immediately after removal and the results, given in Table I, show that an average increase of approximately ¾ lb. resulted from the feeding. The livers were also weighed after chilling overnight, and essentially the same results were obtained although there was some evidence of a slightly greater loss from the livers of fed animals.

pH of Muscles

Table II shows the average final pH (ultimate pH (14)) of the psoas muscle from each treatment. With the exception of Trial I, there was no difference between the muscles from fatigued and normal animals, nor between those from rested and fed animals, but the two latter treatments produced a considerably more acid muscle than did the former. The pH of muscles from fatigued and from normal groups varied widely between pigs but that of rested and of fed animals was relatively uniform.

TABLE II

FINAL pH OF PSOAS MUSCLES

Trial No.	Treatment			
	Fatigued	Normal	Rested	Fed
I	6.64 ±.15	5.99 ±.15	5.73 ±.03	5.47 ±.00
II	5.79 ±.11	5.70 ±.22	5.21 ±.06	5.28 ±.08
III	6.08 ±.28	6.00 ±.34	——	5.54 ±.03

Glycogen and Lactic Acid

The glycogen content of livers from the pigs used in Trial I was determined after storage of the liver at 30°F. overnight, and that of the psoas muscles from Trials II and III was determined within four hours after slaughter. Total extractable acids were determined in psoas muscles from Trial II about eight hours after slaughter, and lactic acid was determined in selected muscles from Trial III 24 hr. or longer after slaughter. The results of these analyses are presented in Table III.

TABLE III

GLYCOGEN AND LACTIC ACID CONTENT OF HOG LIVERS AND PSOAS MUSCLES

Trial No.	Treatment				
	Fatigued	Normal	Rested	Fed	N.D.†
Glycogen (mgm. dextrose /gm.)					
I, livers	0.45‡	0.30	1.26	87.8	49.5
II, psoas	1.26	1.12	2.37	2.75	Not sig.
Acid (mgm. lactic/gm.)					
II, psoas§	7.91	7.88	8.86	9.35	0.81
III, psoas	6.54	7.10	——	7.84	Not sig.

†*Necessary difference for significance between treatments, 5% level.*
‡*Average for two hogs, third had a liver glycogen content of 89.2 mgm./gm. The high N.D. is due to this one anomalous result.*
§*Trial II—total ethanol extractable acids reported as lactic.*

Considerable loss of glycogen probably occurred from both livers and muscles before the analyses were made, but the results are in the expected order. The glycogen content of the psoas muscles shows considerable variation between pigs, and the differences between treatments are not statistically significant. Bate-Smith (4, 5) has found that the muscle glycogen tends to be low and that the carbohydrate reserves of the pigs are more variable than those of the ox.

The acid values shown are essentially "final" values, as glycogen breakdown would be almost complete before the samples were treated. Variability be-

tween pigs was again high, especially in the fatigued and normal groups, but the higher acid content of the muscles having high initial glycogen, i.e., the rested and fed lots, is apparent. A highly significant negative correlation coefficient between final pH and lactic acid content was found (Trial III, 30 paired values, $r = -0.78$).

Chloride Content of Cured Psoas Muscles

The sodium chloride content of the cured muscles is given in Table IV. In Trial I the chloride content of muscles from fed or rested animals was considerably higher than was that from fatigued or normal animals, but in the later trials this effect of the *ante-mortem* treatments was not apparent.

TABLE IV

SODIUM CHLORIDE AND SODIUM NITRITE CONTENTS OF CURED PSOAS MUSCLES

(Wet weight basis)

Trial No.	Sodium chloride in brine,† %	Treatment				
		Fatigued	Normal	Rested	Fed	N.D.‡
Sodium chloride, %						
I	30	6.24	6.32	7.65	8.12	1.29
II	17	5.31	5.94	5.29	4.66	0.81
III	30	6.83	7.15	—	6.96	Not sig.
	20	5.26	5.74	—	5.77	Not sig.
Sodium nitrite, p.p.m.						
II	17	198	199	181	142	40
III	30, 20	80	89	—	55	23

†All pickles contained 1.0% sodium nitrate and 0.05% sodium nitrite.
‡Necessary difference for significance between treatments, 5% level.

Since the pH of the muscles varied with the *ante-mortem* treatments, correlation coefficients of pH with sodium chloride content were determined (Table V). Only one set of data showed a significant correlation, and in view of the wide variation between sets of data little importance can be attached to this single result.

Nitrite Content of Cured Psoas Muscles

The sodium nitrite content of muscles cured as described above is shown in Table IV, and the correlation coefficients of pH with sodium nitrite are included in Table V. In all trials, the sodium nitrite content of the muscles from fed animals was lower than that from fatigued or normal animals, and there was a high positive correlation between the pH of the muscle and the amount of nitrite absorbed from the pickle. The nitrite determinations were all made on the freshly cured and ground muscles.

TABLE V

CORRELATION COEFFICIENTS OF pH WITH SODIUM CHLORIDE AND WITH SODIUM NITRITE CONTENTS OF CURED PSOAS MUSCLES

Trial No.	No. of paired samples	Correlation coefficients	
		pH × sodium chloride	pH × sodium nitrite
I	12	− 0.62	——
II	24	+ 0.20	+ 0.63**
III	12 (20% brine)	− 0.39	
	12 (30% brine)	− 0.12	+ 0.70**
	10 (30% brine)	− 0.65*	

*Significant to the 5% level.
**Significant to the 1% level.

Color of Cured Psoas Muscles

The color of the exposed surface of bacon or ham is an important factor in its quality but is extremely difficult to measure objectively. Attempts to define the color of slices of cured psoas muscle by use of a G.E. recording reflectance spectrophotometer gave results that were of little or no value, owing, apparently, to differences in the surface, the presence or absence of flecks of fat, and the shallow depth (12) of the discolored layer.

Discoloration was greatly increased by grinding the samples, and differences in color stability were thereby accentuated, but physical measurements were difficult owing to the uneven surface. However, Munsell numbers were assigned to the samples by visual comparison with the color charts, and the approximate average ratings given by three operators are shown in Table VI. It is apparent

TABLE VI

pH AND COLOR OF CURED AND GROUND PSOAS MUSCLES

Treatment of pig	pH of psoas, after cure	Color (Munsell No.)
Normal	6.21	7.5 YR 5/4
Fatigued	6.19	5.0 YR 5/4
Normal	5.96	5.0 YR 4/3.5
Fatigued	5.87	5.0 YR 5/3.5
Fatigued	6.01	4.0 YR 4/3.5
Fatigued	5.75	10.0 R 5/3.5
Fed	5.69	9.0 R 5/4
Fed	5.55	7.5 R 5/4
Fed	5.49	7.5 R 5/4

from the data that *ante-mortem* treatment of pigs markedly affected the color stability of the cured meat. Color stability appears to correlate with pH of the meat, and the effect of treatment may be largely the result of altered pH.

Noticeable differences in texture of the meat were also apparent. In the more acid meat the ground particles remained discrete but as the pH increased they became more sticky and at the highest values reported fused together into a viscous mass.

Color of Commercially Cured Meats

The pH of the psoas muscles of the pigs used in Trial III was determined and 12 hogs of widely varying pH were selected for further study. One ham and the bellies from these pigs were cured by a commercial domestic cure and the other ham was cured by the standard Wiltshire method. All cutting and processing was done by plant personnel in a routine operation.

At the end of the curing period the authors classified the 12 pieces from each cure as "good", "medium," or "poor" according to the color of the lean surface. Table VII lists the samples in order of decreasing pH (of the corresponding psoas muscles). The color of the hams was uniform over the whole lean surface, and classification by color was relatively simple. The bellies tended to be somewhat blotchy owing to close contact with other bellies, and grading was difficult. A wide range of color was apparent in each type of produce.

TABLE VII

COLOR OF COMMERCIALLY CURED HAMS AND BELLIES

Final pH of fresh psoas	Treatment of pig	Color rating		
		Hams		Bellies
		Wiltshire	Domestic	
6.48	Fatigued	Poor	Medium	Poor
6.46	Fatigued	Medium	Poor	—
6.32	Normal	Poor	Poor	Poor
6.29	Normal	Poor	Poor	—
6.28	Fatigued	Poor	Poor	Medium
6.23	Fatigued	Medium	Medium	Good
6.01	Normal	Medium	Medium	Medium
5.69	Fed	Medium	Medium	Medium
5.60	Normal	Good	Good	Good
5.51	Fed	Good	Good	Medium
5.47	Fed	Good	Good	Good
5.45	Fed	Good	Good	Good

Freshly cut surfaces of the hams showed no color differences but after a few days at refrigerator temperatures marked differences between hams were apparent. By careful selection of a smoothly sliced, fat-free area it was possible to estimate the color of the ham slices with the "Photovolt" instrument. Munsell notations were calculated from these measurements on five hams and are given, with other relevant data, in Table VIII. The color of the hams from the fatigued pigs was distinctively poorer than that of the hams from fed and normal animals.

The domestic cured produce and the remaining Wiltshire hams were smoked. No color differences were apparent on either freshly cut or exposed surfaces after smoking.

TABLE VIII

pH, COLOR, AND SALT CONTENT OF COMMERCIALLY CURED WILTSHIRE HAMS

Treatment of pig	Final pH of psoas	Cured ham			
		pH	Sodium nitrite, p.p.m.	Sodium chloride, %	Munsell No.
Fatigued	6.48	6.18	104.1	4.76	5 YR 4/2.8
Fatigued	6.28	6.19	106.9	4.99	4 YR 4/2.5
Normal	6.01	6.03	85.5	4.54	7.5 R 4/2.8
Fed	5.69	5.98	74.9	4.63	7.5 R 4/2.8
Fed	5.47	5.95	57.6	5.27	4.5 R 4/3

Microbiological Changes

Psoas muscles from the hogs used in Trial II were put through sterile meat choppers after cure and stored at 30°F. Samples were removed at intervals and the viable count determined. The various *ante-mortem* treatments had little effect on the rate of growth or on the final number of organisms (Table IX).

TABLE IX

BACTERIAL CONTENT OF GROUND PSOAS MUSCLES AFTER STORAGE AT 30°F.
(Counts in thousands per gram; average values of duplicate determinations on six samples)

Treatment of pig	Initial	Days				
		4	12	20	35	55
Fatigued	17	14	11	46	41	19,000
Normal	22	22	23	17	78	12,000
Fed	61	32	20	—	140	19,000
Rested	14	14	10	—	27	4600

In Trial III, pairs of psoas muscles having approximately the same pH (Table X) were cured and then ground aseptically. In two samples there was a definite increase in pH during curing and storage, but in the majority the change was very slight. The total viable count tended to decrease as the pH decreased. However, as the samples with the higher pH had an odor different from that of the more acid meat, and as the colonies on the plates had a different appearance, all of the colonies (approx. 30) in a sector of the final plates were examined morphologically. The flora consisted entirely of yeasts and cocci, and the proportion of yeasts increased as the pH decreased. The viable bacterial counts calculated from the total viable count using the percentages shown in the table indicated that bacterial development was reduced drastically between pH 6.0 and 5.7 (final pH). This is in close agreement with the findings of Ingram (19) for pure cultures of bacteria isolated from spoiled hams.

It is difficult to reproduce commercial conditions in the laboratory, and the bacterial flora might not be representative. To determine whether the flora of

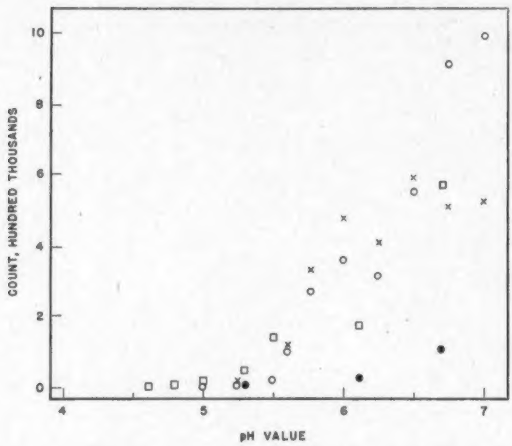


FIG. 1. *Effect of pH of medium on the relative numbers of microorganisms on four lots of Wiltshire sides. Acetate and phosphate buffers—open circles and crosses; McIlvaine's buffers—closed circles and squares.*

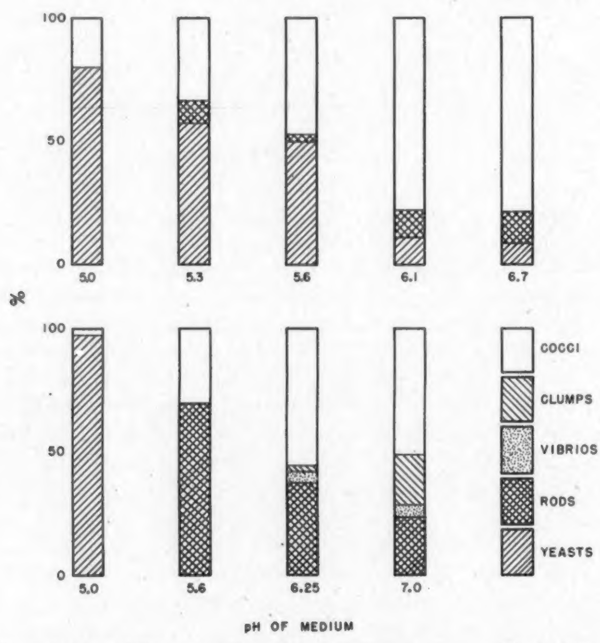


FIG. 2. *Percentages of different groups of organisms growing on media of different pH. Top row—McIlvaine's buffers; bottom row—acetate and phosphate buffers.*

TABLE X

CHANGES IN BACTERIAL NUMBERS AND FLORA IN GROUND PSOAS MUSCLES STORED AT 30°F. FOR 35 DAYS, THEN AT 40°F. FOR 20 DAYS

Treatment of pig	pH		Log total viable count, 55 days	Log viable bacterial count, 55 days	Bacterial increase	Cocci, %	Yeast, %
	Before curing	After curing and storing					
Fatigued	6.38	6.33	9.43	9.41	2.7×10^{9}	96.7	3.3
Fatigued	6.33	6.35	8.65	8.56	3.6×10^{8}	80.0	20.0
Rested	6.15	6.13	8.36	8.14	1.4×10^{8}	60.0	40.0
Fatigued	6.03	6.28	8.49	8.09	1.2×10^{8}	40.0	60.0
Rested	5.61	6.01	7.91	7.52	3.3×10^{7}	40.0	60.0
Fatigued	5.65	5.70	6.09	4.60	2.6×10^{2}	3.3	96.7
Fed	5.50	5.58	7.64	0	0	0	100.0

Wiltshire sides responded similarly to pH, rib and cut surfaces of commercially cured sides were swabbed and, after appropriate dilution, plates were poured using agar adjusted to various pH levels. In the first test, acetate buffers were used to adjust the medium from pH 5.5 to 5.0 and phosphate buffers from 5.6 to 7.0. There was a definite decrease in the viable count as the pH decreased (Fig. 1), though the more drastic reduction at pH 5.5 and below may have been partially due to the acetate buffer and not to the effect of pH alone. There was also a definite change in the bacterial flora with pH (Fig. 2).

In the second test using McIlvaine's buffers similar trends were noted but a much greater decrease in bacterial numbers occurred between pH 6.7 and 6.0. Here again there was a definite change in the flora (Fig. 2) although yeasts persisted at the higher pH levels and the proportion of rods showed little change.

Discussion

Although the Canadian bacon pig has been bred for the production of a uniform side of bacon its physiological–biochemical characteristics are highly variable. This variability stems from the congenital variability of living organisms augmented by differences in age, diet, daily exercise, level of diet during preslaughter period, environmental conditions, method, distance, and possibly season of shipment, etc., and it is not surprising that differences of the type investigated in these studies are somewhat masked by the random differences between pigs. Nevertheless, when considered together with the known effects of feed and fatigue on animals (9, 10, 23) there can be little doubt as to the physiological significance of the differences noted.

The data presented in this paper, together with the published data of others (7, 14), make it apparent that the pH of the pork going into cure is an important factor in determining the quality of the product. The data of Tables IV and V do not support the claim (13) that chloride penetrates into rested muscle (low pH) more readily, but the conditions used in the present experi-

ments did not permit fine distinctions to be made between treatments. The amount of chloride penetrating into a muscle must also be affected by the surface–volume ratio of the muscle and this would be drastically altered by the tears and fissures which occurred in some muscles.

Earlier workers in these laboratories (22) found that in muscles in which the pH had been adjusted artificially, water loss was less but salt penetration greater in the more alkaline samples. Callow (13) has postulated that salt penetrates more easily into rested muscle owing to a more open structure, but the open structure or water-cell relations may not be controlled by pH, since it has been shown (15) that rate of cooling has an effect on the structure but not on the pH of the muscle. This may explain in part the effects obtained here, since in Trial I the psoas muscles were cooled with the carcasses whereas in subsequent trials the muscles were removed from the hot carcasses and cooled at 30°F.

The data of Table IV show a distinct difference in the amount of nitrite found in freshly cured meats, and these differences were positively correlated with the pH (Table V). The manner in which pH affected the nitrite content is not apparent, but since 0.05% sodium nitrite was present in the curing pickle, and the muscles were cured for only 24 hr., the effect would not appear to be related to differences in the rate of bacterial reduction of nitrate. The low nitrite content of the more acidic muscles may result from a greater destruction of nitrite by reaction with amino groups.

The color of the cured product is an important quality characteristic, and Canadian bacon has been criticized frequently on the British market because cut surfaces became brown and unattractive. The data of Tables VI, VII, and VIII show that the red color is more stable in meat of low pH. This effect is the reverse of that noted in solutions of nitric oxide hemoglobin (25), or of hemoglobin (11), but the pH range of muscle extends further to the acid side than did that of the solutions studied. There appears to be a sharp decrease in the rate of methemoglobin formation between pH 5.9 and 5.5.

The data presented in Tables IX, X, and in Figs. 1 and 2 show that bacterial growth was considerably reduced if the pH of the meat was below 5.6. The development of yeasts, which occurred below this pH, does not lead to rapid spoilage, as these organisms are not strongly proteolytic and do not give rise to the characteristic offensive odors of spoiled meat. It is thus apparent that the keeping time of the meat will be considerably extended if the pH is low.

The correlation between pH and lactic acid content of the muscle suggests that lactic acid is the most important single factor influencing the pH. The buffering capacity of the meat will also influence the pH, but probably does not differ very greatly with different degrees of fatigue (6, 7).* The

**Bate-Smith (7) has suggested a third factor which he terms the "basal" pH, but acceptance of this suggestion must await presentation of further supporting data.*

chief source of lactic acid is carbohydrate, and the problem of obtaining meat of low pH becomes primarily one of maintaining a high reserve carbohydrate (glycogen) level in the muscles.

It can be assumed that glycogen reserves in the muscles of a pig may be destroyed, (*a*) by aerobic oxidation during gentle exercise, without accumulation of lactic acid, (*b*) by anaerobic metabolism during relatively violent exercise, with the production of lactic acid that will be largely lost to the blood and liver, and (*c*) by anaerobic postslaughter metabolism, with the production of lactic acid which is retained by the muscles. The pH of the muscle several hours after slaughter will depend upon the proportion of glycogen consumed by the three metabolic pathways as well as upon the original supply.

The average haul, by rail or truck, required to move Canadian pigs to market is sufficient to preclude the possibility of their arriving at the plant in a rested condition, i.e., with high muscle glycogen reserves. Rest alone brings about recovery of these reserves very slowly if at all (17, 20) and for practical reasons long holding periods are undesirable. However, when sugar is fed there is a rapid deposition of glycogen in the muscles and liver, and a portion of the cost of the sugar is offset by the increased weight of liver obtained. Further investigation is needed to determine the optimum time between feeding and slaughter, and also to determine the efficiency of other feeds for this purpose.

Having established adequate glycogen reserves some precautions against its destruction are required. The energy expenditure required for walking from the barns to the shackling pen is often considerable, and Callow (16) points out that walking a quarter mile will produce detectable differences in the psoas muscles. In most animals the glycogen lost by this amount of exercise would not be serious provided aerobic metabolism was maintained. The ability of the pig to supply this energy by strictly aerobic means will depend upon the rate at which he is driven and upon his previous "athletic" training, and the latter factor varies extensively with Canadian pigs. Also, Bate-Smith (4, 5, 7) has drawn attention to the "special" metabolism of pigs and this suggests that their muscle glycogen may be depleted by muscular activity more easily than that of other animals.

The effect of the death struggles on muscle glycogen and lactic acid is difficult to estimate. It has been shown (2) that a single twitch of a rat's leg causes detectable glycogen loss, and a considerable destruction of glycogen must therefore occur between the "stick" and the relaxation of the muscles with death of the animal. However, much of the lactic acid formed at this time may be retained in the muscles, as the blood supply is rapidly depleted. The initial lactic acid values found in the present work are high, and this may have been due to the anaerobic breakdown of glycogen during the death struggles as well as during the period between slaughter and sampling.

It should be emphasized that the quality characteristics which are markedly improved by proper preslaughter treatment of the animal are largely those

required for the British market. Bacon for domestic consumption is usually smoked, and this procedure stabilizes the color and prolongs the keeping time sufficiently for normal marketing. Wiltshire sides, on the other hand, are exported "green" and a large proportion is retailed on the British market unsmoked. Feeding, resting, and careful handling of pigs destined for the production of "Wiltshires" would result in a considerable improvement in the uniformity and quality of the product and would be highly desirable.

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NOTES

Antioxidant Properties of an Ethylene Dichloride Extract of Cottonseed Meal[1]

The recommended use of ethylene dichloride for extraction of wheat-germ oil (4) and demonstration of the antioxidant properties of the oils so obtained (7) suggested that this solvent might be useful in removing antioxidants from other natural sources. Cottonseed meal was used because it has been shown to yield potent fat-stabilizing materials on extraction with an acetic acid—acetone mixture (2).

Hot-pressed cottonseed meal, ground to 100 mesh in a Wiley mill and extracted with ethylene dichloride, yielded 6% of a dark brown, viscous oil. Measurements (3, 5, 8) showed that this oil had an iodine value of 102, a phosphorus content of 0.2% (corresponding to about 5% lecithin), and contained no tocopherols. Gossypol was presumably absent (1). Tests by the Swift stability method at 97.8° C. (7) indicated that the ethylene dichloride extract stabilized lard and was more active than corresponding amounts of wheat-germ oil. For example, the stability time of a control lard (175 min.) was increased by 60 and 120 min. by the addition of 0.05 and 0.1% of wheat-germ oil, respectively, and by 170 and 440 min. by addition of the same percentages of the cottonseed extract.

Further extraction of cottonseed meal (already extracted with ethylene dichloride) with 3% acetic acid in acetone yielded 1% of a dark brown, sticky gum that was almost insoluble in ether. This material, similar to that described elsewhere (2), also had a definite stabilizing action on lard. It was more effective than wheat-germ oil, but less effective than the initial ethylene dichloride extract of the meal, i.e., the stability of a control lard (180 min.) was increased 55 min. by 0.05% wheat-germ oil, 155 min. by 0.05% ethylene dichloride extract, 110 min. by 0.05% acetic acid - acetone extract, and 195 min. by 0.1% acetic acid - acetone extract. The activity of such acetic acid - acetone extracts has been considered to be due to the presence of a basic oxygen compound (2).

The ethylene dichloride extract was about 95% soluble in ethyl ether and about 25% soluble in absolute methanol. Swift stability tests in lard of the soluble and insoluble fractions (in proportion to their presence in the original material) indicated that most of the antioxidant activity was in the soluble fractions. Stability of control lard (205 min.) was increased 545 min. by 0.1% cottonseed extract, 455 min. by 0.094% ether-soluble fraction of extract, 15 min. by 0.006% ether-insoluble fraction of extract, 200 min. by 0.025%

[1] *Excerpt, in part, from a Ph.D. thesis submitted to McGill University in 1944. Issued as Paper No. 251 of the Canadian Committee on Food Preservation.*

methanol-soluble fraction of extract, and 125 min. by 0.075% methanol-insoluble fraction of extract.

Five per cent of the ether-soluble fraction was precipitated by adding acetone. The acetone-insoluble fraction thus obtained was considerably more active than the acetone-soluble material that remained, since stability of control lard (150 min.) was increased 200 min. with either 0.095% acetone-soluble fraction of the extract or 0.005% acetone-insoluble fraction of the extract. The stabilizing power of the acetone-insoluble fraction was not increased by citric acid. Stability of control lard (210 min.) was increased 395 min. by 0.01% of acetone-insoluble fraction, and 390 min. by 0.01% acetone-insoluble fraction *plus* 0.002% citric acid.

The lack of synergism with citric acid (6) suggests that the antioxidant effect of the ethylene dichloride extract was largely due to phosphatide. This assumption is supported by the solubility relations, the appreciable phosphorus content of the original extract, and the absence of other known stabilizers. The ethylene dichloride extracts of cottonseed meal appear to be largely residual cottonseed oil containing a relatively high concentration of phosphatides, which are chiefly responsible for the antioxidant properties demonstrated.

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